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C2U 3 4C4A 4C5 4D1 4DX 4N1 6A1 8A1  
A5B 170 180 240 242 243 24Y H  
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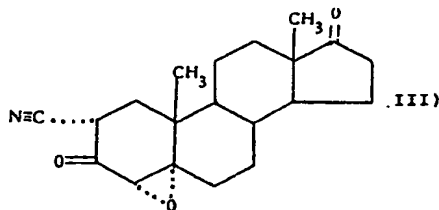
GB 1123770  
Chemical and Pharmaceutical Bulletin Volume 29 No  
9 1981 pages 2478-2484 Chemical and  
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2646-2652 Chemical Abstracts 93 215759 K

(58) Field of search

C2U

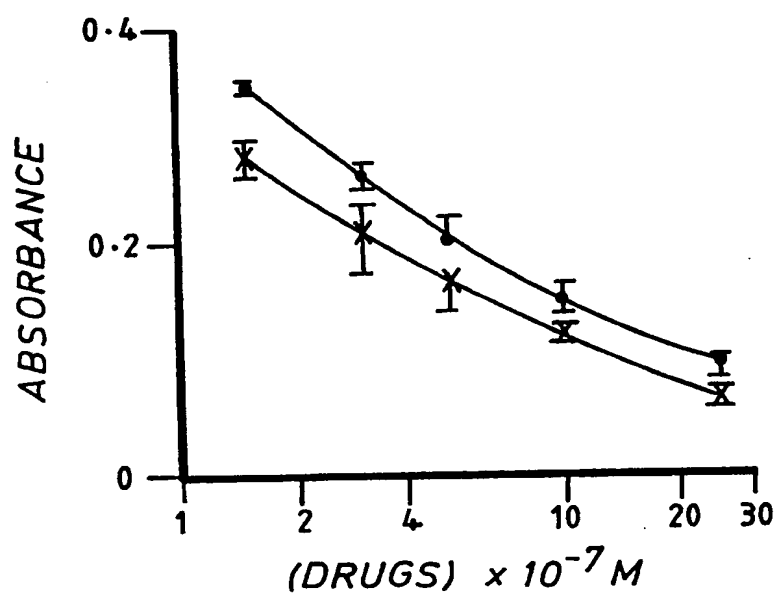
(54) **2 $\alpha$ -Cyano-4 $\alpha$ ,5 $\alpha$ -epoxy-androstane-3,17-dione**

(57) The title compound of formula (III) inhibits adrenal steroidogenesis, and is more active than the corresponding 17 $\beta$ -ol (trilostane). It is prepared by ring-opening of the corresponding [2,3-d]isoxazole.

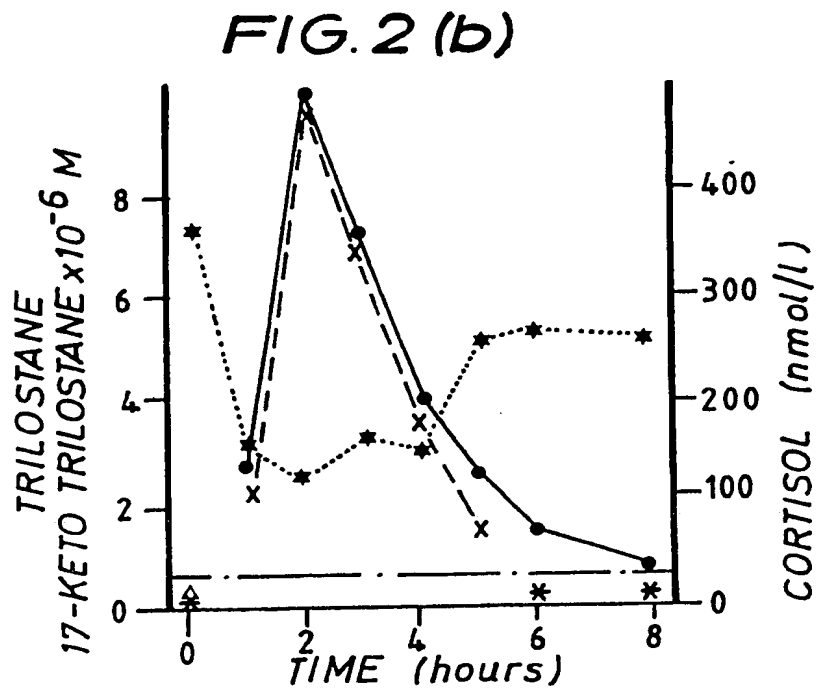
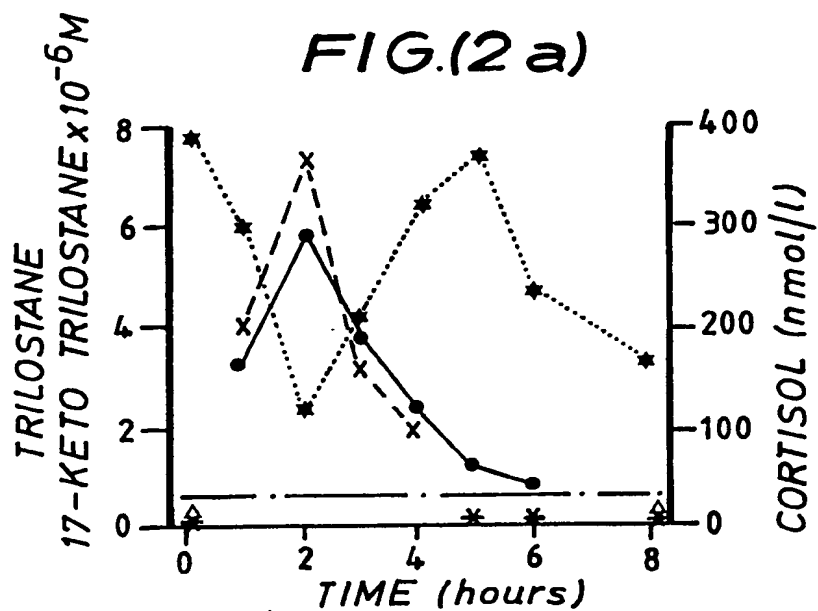


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FIG. 1



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FIG. 2(c)

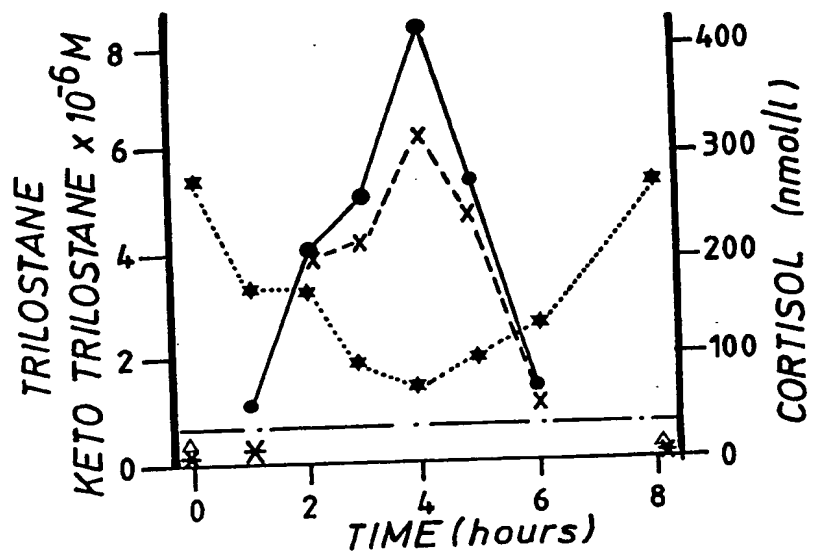
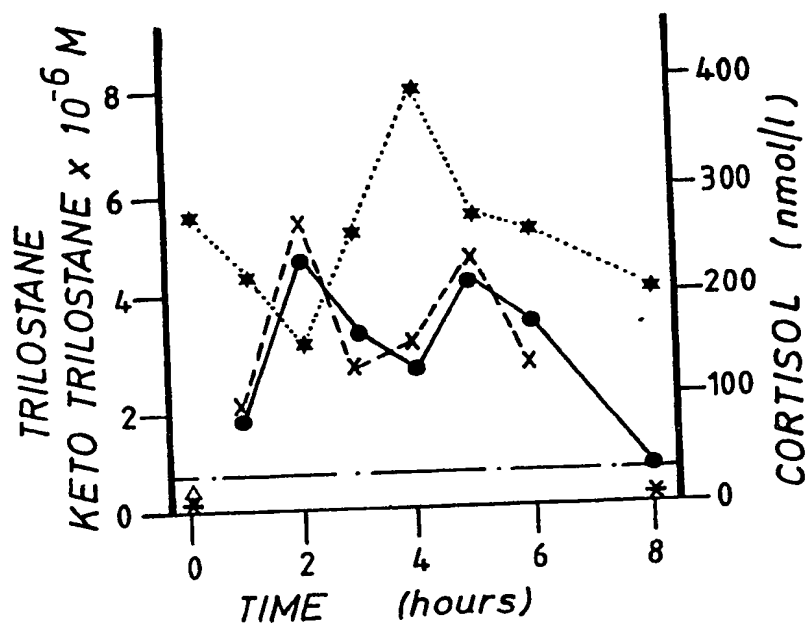


FIG. 2(d)



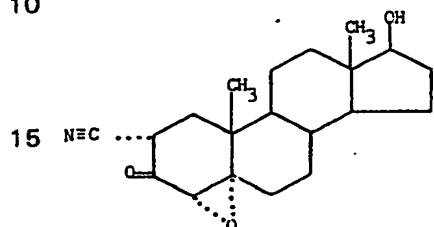
## SPECIFICATION

## Steroid compound

- 5 The present invention relates to a new pharmaceutically-active steroid compound and, in particular, to a metabolite of the compound having the common name "trilostane", which metabolite has significantly higher activity than the parent compound.

The compound "trilostane", which is 2 $\alpha$ -cyano-4 $\alpha$ ,5 $\alpha$ -epoxyandrostan-17 $\beta$ -ol-3-one having the formula:

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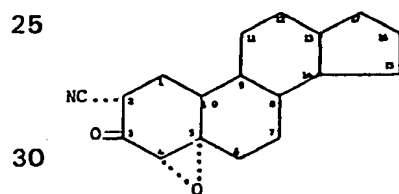


I

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- 20 is described, along with related compounds in, for example, British Patent Specification No. 1,123,770 and U.S. Patent Specification No. 3,296,295. Those earlier specifications describe the adrenocortical inhibiting properties of trilostane and related compounds, which generally have a ring structure represented by the following formula:

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II

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- In the earlier disclosures, it is said that the exact nature of the steroid moiety is not critical, and it can be derived from any steroid of the general type known to exhibit hormonal or other pharmacological or endocrinological properties. Such steroid moieties are said to have from 19 to about 23 carbon atoms, not counting carbon content which may be provided by esterified hydroxy groups.

- In particular, representative of the steroid moieties which are said to make up compounds of the earlier invention as disclosed in the British Specification are those having, at position 17, a hydroxy, acyloxy or oxo group or both a hydroxy and a lower alkyl or lower alkynyl group, as characteristic of the androgenic and anabolic steroids. The steroid moiety also is said to be one which can have one or more substituents at other positions of the nucleus, for example, hydroxy, ethyloxy or oxo groups at positions 6, 7, 11, 12, 14 (presumably "15" not "14" is intended) or 16; epoxy groups at adjacent positions, for example, at the 9,11-, 11,12- or 15,16- positions; halogen atoms, preferably fluorine, chlorine or bromine, for example, at the 6-, 7-, 9-, 12-, 16 or 17- positions; and lower-alkyl groups, for example, at the 6-, 7-, 11- or 16- positions. Furthermore, the steroid moiety is said to be one which can have one or more double bonds, for example, at the 6,7-, 9,11- or 16,17- positions, and the steroid moiety can also possess angular methyl groups at C<sub>10</sub> and C<sub>13</sub>. Although in general terms the disclosure of the basic U.S. Specification is somewhat broader than that of the British Specification, for example, in that it is not limited to epoxy compounds, the same or similar considerations generally apply to the U.S. disclosure.

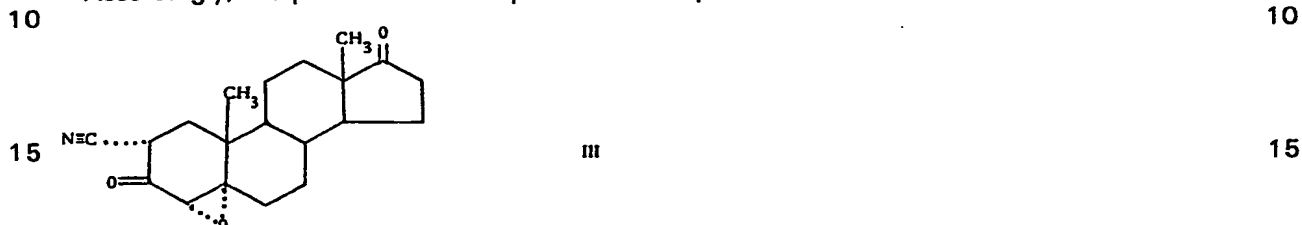
- In published studies on the metabolism of trilostane, for example, by Mori et al in Chemical and Pharmaceutical Bulletin (Tokyo), 1981, 29 (9), pages 2478 to 2484 and pages 2646 to 2652, and by Suzuki et al in Proceedings of the 11th Symposium on Drug Metabolism and Action, November 6-7, 1979, pages S-10 and S-22, earlier workers have identified *inter alia* a metabolite of trilostane in which the above formula I the hydroxy group at the 17-position is replaced by a keto group. In the earlier published studies, the 17-keto metabolite is identified merely as one metabolite amongst a number of metabolites, and no information is given as to whether or not that or any other metabolite has any activity of itself.

- While recognising that the 17-keto metabolite of trilostane is embraced within the generic disclosure of the above-mentioned earlier specifications, although it is not specifically disclosed therein, we believe that until now it has been thought that the metabolites of trilostane do not have the activity of the parent compound. Accordingly, it has previously been thought desirable to choose a form or means of administering trilostane which achieves the lowest possible ratio of

17-keto metabolite to parent compound, so that the patient receives maximum benefit from the active parent compound, and oxidation to the 17-keto metabolite and associated products is avoided for as long as possible.

Contrary to the above thinking, we have now found quite surprisingly that the 17-keto metabolite of trilostane has a relative *in vitro* potency compared with the parent compound of about 1.7 times as high as the parent compound on a weight for weight basis. Thus, we believe the 17-keto metabolite is the major active component when trilostane is administered for clinical purposes, rather than the compound *per se*.

Accordingly, the present invention provides a compound of the formula:



for use in a method of treatment of the human or animal body by therapy, in particular therapy to modulate or inhibit adrenal steroidogenesis, typically in any treatment requiring the administration of an adrenocortical inhibitor.

As will be appreciated by those familiar with the parent compound trilostane, the compound of the above formula III may be considered to exist in the 3-keto form shown and/or in the corresponding 3-enol form. It is to be understood, therefore, that wherever the keto form is shown or referred to herein and in the claims it is intended to embrace also the corresponding enol form or any mixture of the two forms.

The invention also provides a pharmaceutical composition, which composition comprises a compound of the above formula III, optionally together with one or more other active compounds, and including one or more pharmaceutically-acceptable excipients or carriers.

Such excipients or carriers may be solid, semi-solid or liquid as appropriate to the pharmaceutical form chosen, and may include a wide range of organic and inorganic solids and semi-solids, as well as aqueous and non-aqueous liquids. Of such materials, there may be used, for example, talc, gum arabic, starch, lactose, magnesium stearate or fatty substances of animal or vegetable origin such as cocoa butter, lanolin derivatives, paraffin derivatives or glycols. In addition, the excipient or carrier may be compounded with one or more wetting, dispersing or emulsifying agents and/or one or more preservatives as desired.

By way of example, the compositions of the invention may be presented as a tablet, a capsule, a granulate for suspension, a cream, an ointment, a suppository, a solution or a suspension. Preferably the composition of the invention includes a solid excipient or carrier with which the compound of formula III is mixed or co-granulated, for example, starch, lactose and/or magnesium stearate. More preferably, the composition is presented as a tablet or in an encapsulated form, and in the latter case the compound of formula III, together with any excipient or carrier is filled into the shell of a capsule.

Typically, the composition of the invention when in unit dosage form may comprise a unit dose of from about 30 to about 250 mg of the compound of formula III. The unit dose levels employed may be the same as or less than the corresponding unit dose levels for the parent compound, trilostane, depending on the regimen required compared with the parent compound. Thus, the compound of formula III may be administered at comparable dose levels to the parent compound, that is to say at a unit dose of from about 50 to about 250 mg, for example, about 60 mg, about 120 mg and about 240 mg, and more preferably from about 50 to about 120 mg, or at lower unit dose levels, for example, below about 100 mg.

As disclosed in our co-pending Application No. 83-29173, the parent compound trilostane is particularly effective when administered as a compound in particulate form consisting of particles having a mean equivalent sphere volume diameter of less than about 20 microns, at least 95% of the particles having a particle size of less than about 50 microns. The compound of the present invention also is preferably prepared and administered in a form having a reduced particle size, which more preferably is either one within the parameters laid down in the specification of the above-mentioned earlier Application with respect to particle size measured as a mean equivalent sphere volume diameter as generally defined above, and in particular from about 5 to about 10 microns, or one within the additional or alternative particle size parameters expressed in terms of a specific surface area, preferably of about  $2\text{m}^2\text{g}^{-1}$  or higher, more preferably from about 2 to about  $5\text{m}^2\text{g}^{-1}$  e.g. from about 2 to about  $4\text{m}^2\text{g}^{-1}$ .

If desired, the active compound of the above formula III may be employed in admixture with one or more other active compounds such as trilostane *per se*.

As generally indicated above the compound of the invention can be used in the treatment of

adrenal cortical hyperfunction as in hypercortisolism and primary aldosteronism. Thus, for example, it may be used in the treatment of Cushing's Syndrome and Conn's Syndrome, and also in the treatment of breast and prostate carcinoma.

The enhanced activity of the 17-keto metabolite of formula III above compared with trilostane itself can be seen from studies carried out as described below.

In the description drawings in which:

Figure 1 is a graphical representation showing the relative inhibition of rat ovarian  $3\beta$ -hydroxysteroid dehydrogenase activity by trilostane and the 17-keto trilostane metabolite of formula III, and

Figures 2(a) to 2(d) are graphical representations showing total plasma trilostane and 17-keto trilostane metabolite vs. trilostane-like bioactivity and plasma cortisol in four subjects studied.

In a first study, a cytochemical bioassay was employed to determine the relative potency of the 17-keto trilostane metabolite versus trilostane, in three experiments. From the results obtained, standard curves covering the range from 0.15 to 2.5  $\mu$ M were prepared for the metabolite and the parent compound. Potency estimates were calculated by computer analysis of the relative slopes and errors around each dose point.

The cytochemical bioassay is based upon the reaction, within serial unfixed tissue sections of the dioestrous rat ovary, between the enzyme,  $3\beta$ -hydroxysteroid dehydrogenase —  $\Delta^5$ -3-oxosteroid isomerase ( $3\beta$  HSD) contained therein, and added substrate, cofactors and a colourless tetrazolium salt. The reaction leads to the deposition of an intensely coloured blue insoluble formazan dye in the sections, and the amount of dye produced within a section over a given period (absorbance) is measured by means of a scanning integrating densitometer. If an inhibitor of  $3\beta$  HSD, such as trilostane or 17-keto trilostane metabolite is added to the system, the amount of blue dye formed will be reduced. The more active the compound or the larger the amount added, the greater will be the reduction in absorbance.

The bioassay curves obtained are as shown in Fig. 1 of the accompanying drawings, results being expressed as the mean  $\pm$  SD ( $n = 4$ ). As can be seen from Fig. 1, both trilostane (upper curve—black circles) and the metabolite (lower curve—crosses) exhibit parallel dose response lines over the range of 0.15 to 2.5  $\mu$ M. However, the fact that the curve for the metabolite is lower than that of trilostane indicates that the metabolite inhibits the enzyme to a greater extent than trilostane on a weight for weight basis, that is to say the metabolite is more active. In fact, as can be calculated from Fig. 1, the metabolite is 1.71 times (95% confidence, 1.4–2.1) more active on a weight for weight basis than the parent molecule.

In a second study, trilostane as 2  $\times$  60 mg capsules was taken orally by 4 healthy male volunteers between 0900 and 1000 hours each morning and the volunteers were fasted. A heparinised blood sample was taken before dosing and at various times up to 8 hours after dosing. Plasma was prepared and 2 to 5 ml aliquots were snap frozen and stored at  $-20^\circ\text{C}$ .

Trilostane and its 17-keto metabolite were extracted from acidified plasma by a simple chloroform extraction procedure. In each case, one portion of the extract was taken for analysis by high pressure liquid chromatography (h.p.l.c.), and a second duplicate portion was taken for cytochemical bioassay as described above. Using those extracted portions, plasma trilostane and 17-keto metabolite concentrations were separately estimated by h.p.l.c. assay, and plasma trilostane-like bioactivity was measured by cytochemical bioassay. In addition, plasma cortisol was assayed by radioimmunoassay using the Amerlix Kit (obtained from Amersham International Amersham, Buckinghamshire).

Since the bioactivity recorded relative to a standard curve for trilostane was substantially higher than the molar sum of circulating trilostane and 17-keto compound as assessed by h.p.l.c., the h.p.l.c. figures were corrected for a 1.71 times higher activity in the 17-keto compound, and the results plotted concentration vs. time as shown in Figs. 2(a) to 2(d) of the accompanying drawings for each of the volunteer subjects. In Figs. 2(a) the plots shown have the following key:

1. Black star positions—plasma cortisol (nmol/litre)
2. Black circle positions—molar sum of trilostane and 17-keto trilostane (times 1.71 to allow for increased potency) as determined by h.p.l.c.
3. Crosses—trilostane-like bioactivity as determined by cytochemical bioassay.

(The lower limit of detection of the cytochemical bioassay is  $1 \times 10^{-6}$ M: that for the h.p.l.c. assay is  $5 \times 10^{-7}$ M. This is indicated by the horizontal dot/dash line below which are experimental points—triangles for h.p.l.c. and stars for cytochemical bioassay—where trilostane and 17-keto metabolite were undetectable).

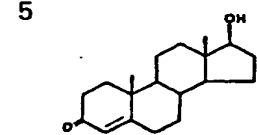
As can be seen from Figs. 2(a) to 2(d), the agreement between the bioassay of trilostane-like activity (crosses) and the molar sum of trilostane and 17-keto trilostane (times 1.71 to account for its increased potency) assayed by h.p.l.c. (black circles) is excellent.

In addition, a notable feature of the profiles shown is the clear relationship between the concentration of total active drug and cortisol in the plasma. Thus, where there are clear sustained peaks of trilostane and 17-keto metabolite, cortisol secretion is clearly shown to be

suppressed and cortisol secretion recovers only when the drug levels decline.

The compound of the invention of the above formula III may be prepared by a variety of synthetic routes, in particular starting either from testosterone, that is a compound of the formula:

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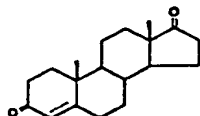


IV

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or from androstenedione, that is a compound of the formula:

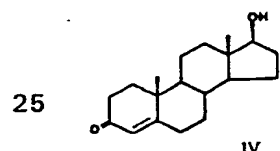
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V

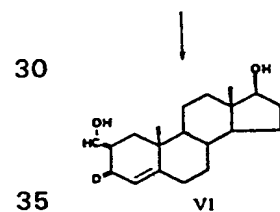
The preparative routes which are preferably followed can be summarised by the following reaction scheme:

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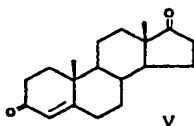
IV

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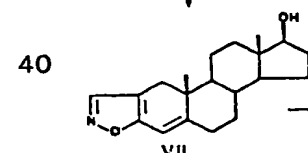
VI

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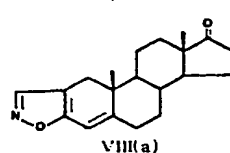
V

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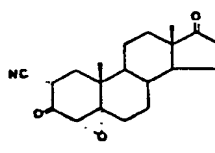
VII

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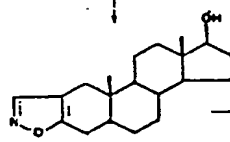
VIII(a)

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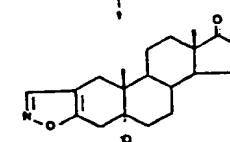


III

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VIII(b)



IX

In accordance with the above reaction scheme the compound of the above formula III is preferably prepared by one of the following two preparative routes:

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#### 1. First Preparative Route:

17 $\beta$ -hydroxyandrost-4-en-2-one of formula VII is oxidised to 17-ketoandrost-4-  
 eno[2,3-d]isoxazole of formula VIII(a), which is then epoxidised to 17-keto-4 $\alpha$ ,5 $\alpha$ -epoxyandros-  
 60 eno[2,3-d]isoxazole of formula IX, from which the compound of formula III is obtained by  
 opening the isoxazole ring. For this route the starting material of formula VII may be prepared,  
 for example, by reacting a 2-(1-hydroxyalkylidene)-3-oxo compound of formula VI with hydroxy-  
 65 3,135,743. In turn, the compound of formula VI may be obtained from testosterone (compound 65





As well as the above-described steps, the processes of the invention may include one or more further steps of bringing the thus-prepared compound into a desired particle size form, for example, as described in co-pending Applications Nos. 83-29173 and 84-27300. Additionally or alternatively, the processes may include one or more further steps of blending the compound of the invention with one or more pharmaceutically-acceptable excipients or carriers, and optionally thereafter bringing the thus-formed composition into a unit dosage form, for example, as a tablet, a capsule, a granulate for suspension, a cream, an ointment, a suppository or a suspension. In particular, the processes of the invention may include the additional steps of granulation and terminal blending subsequent to isolation of the compound of the invention, e.g. in a reduced particle size form.

The compound prepared in the desired form by one of the above routes may be isolated from the reaction mixture, by for example, filtration, then washed and dried. A variety of drying techniques may be employed which may include one or more of suction drying, tray drying and oven drying. In addition, as may be necessary, one or more of the various steps of the processes of the invention may be carried out under an inert atmosphere e.g. under an atmosphere of nitrogen.

The routes for the preparation of the compound of the invention, together with formulations containing the compound, are illustrated in the following specific Examples.

## 20 Example 1 20

### A. First Preparative Route

#### a) 17-ketoandrost-4-eno[2,3-d]isoxazole [compound VIII(a)]

To a stirred suspension of 106 g of pyridinium chlorochromate in 850 ml of dichloromethane, cooled in an ice bath, was added a solution of 100 g of 17 $\beta$ -hydroxyandrost-4-eno[2,3-d]isoxazole (compound VII) over a period of 1.5 hours. The temperature of the reaction mixture was maintained at approximately 15°C. After the addition was complete, the reaction mixture was stirred for a further 2 hours, then the dichloromethane solution was decanted from the black tarry residue and the residue was extracted twice with hot dichloromethane. The dichloromethane extractions were combined and washed twice with 500 ml of 2M hydrochloric acid. The organic layer was then slurried twice with 7 g of silica gel to give a pale green solution. The solution was evaporated to dryness and the solid residue was slurried with methanol, collected by filtration and the residue washed with a little methanol. A small sample was removed and recrystallised from dichloromethane to give a sample m.p. of 251 to 253°C. The remainder (65.5 g) was used directly in the following preparation.

#### b) 17-keto-4 $\alpha$ ,5 $\alpha$ -epoxyandrostano[2,3-d]isoxazole (compound IX) 35

To a stirred solution of 65.5 g of 17-ketoandrost-4-eno[2,3-d]isoxazole [compound VIII(a)] in 650 ml of dichloromethane were added portions of 43.6 g of metachloroperbenzoic acid over a period of 20 minutes. The reaction mixture was cooled to keep the temperature below 20°C. When the addition was complete, the reaction mixture was stirred overnight at room temperature, then the solids were removed by filtration and the residue was washed with a little chloroform. The combined filtrates were washed with dilute sodium bisulphite until a negative starch-iodide test was obtained, after which the organic layer was washed twice with dilute sodium bicarbonate solution and finally with a saturated brine solution. The organic layer was next dried over anhydrous magnesium sulphate, evaporated to dryness *in vacuo*, after which the solid residue was slurried, with ice cooling, in methanol. The solid was finally collected by filtration and dried under suction.

The filter cake was recrystallised from dimethylformamide, the crystals were collected by filtration under reduced pressure, washed with cold methanol, and then dried overnight at 50°C *in vacuo*. Yield 41 g m.p., 276°C (with decomposition).

#### c) 2 $\alpha$ -cyano-4 $\alpha$ ,5 $\alpha$ -epoxyandrostano-3,17-dione (compound III)

To a stirred suspension of 41 g of 17-keto-4 $\alpha$ ,5 $\alpha$ -epoxyandrostano-[2,3-d]isoxazole (compound IX) in 1 litre of tetrahydrofuran were added portions of 19.5 g of sodium methoxide, over a period of 10 minutes. The reaction mixture was stirred at room temperature for 22 hours. After this time water was added to the reaction mixture and most of the tetrahydrofuran was removed by evaporation under reduced pressure. More water was added to give a suspension of the sodium salt. The suspension was acidified by the slow addition of a 1:1 solution of hydrochloric acid in water. The almost white solid was collected by filtration under reduced pressure, and the solids were washed with water and dried *in vacuo* for 2 hours. The solid was recrystallised from ethanol and dried at 50°C *in vacuo* overnight. Yield 31 g, m.p. 236 to 238°C (with decomposition).

### Microanalytical data

	Calculated	Found
% C	73.37	73.13
5 % H	7.70	7.84
% N	4.28	4.19

In addition, the 17-keto structure was confirmed by infra red and nuclear magnetic resonance  
10 spectral analysis. 10

### Example 2

#### B. Second Preparative Route

##### a) 17-keto-4 $\alpha$ ,5 $\alpha$ -epoxyandrostando[2,3-d]isoxazole (compound IX)

15 To a stirred suspension of 15.1 g of pyridinium chlorochromate in 500 ml of dichloromethane  
cooled to 2°C in an ice-water bath was added a solution of 15.4 g of 17 $\beta$ -hydroxy-4 $\alpha$ ,5 $\alpha$ -  
epoxyandrostando[2,3-d]isoxazole [compound VIII(b)] in 250 ml of dichloromethane over a period  
of 50 minutes. The reaction was stirred at 2°C for a further 16 hours, when the suspended  
solids were removed by filtration under reduced pressure, and the filtrate was extracted twice  
20 with 2M hydrochloric acid. The organic layer was dried over anhydrous sodium sulphate and 20  
g of silica gel were added to the dry organic layer. The excess solvent was removed under  
reduced pressure to give the solid reaction mixture supported on free flowing silica. The product  
was obtained from the silica by elution with a solvent which comprised 10% ethyl acetate in  
dichloromethane and collection of fractions. The fractions which contained the desired product  
25 were combined and evaporated to dryness to give a white crystalline material. Yield 12.8 g,  
m.p. 233 to 234°C (with decomposition). 25

##### b) 2 $\alpha$ -cyano-4 $\alpha$ ,5 $\alpha$ -epoxyandrostan-3,17-dione (compound III)

30 To a stirred suspension of 12.8 g of 17-keto-4 $\alpha$ ,5 $\alpha$ -epoxyandrostando-[2,3-d]isoxazole (com-  
pound IX) in 400 ml methanol were added 200 ml of methanol in which had been dissolved  
1.5 g of sodium metal. The reaction mixture was stirred overnight. The clear solution was  
evaporated to half its original volume under reduced pressure and 300 ml of water and 170 ml  
of 1M hydrochloric acid were added and the resulting suspension stirred for 0.5 hours. The  
solids were collected by filtration and then air-dried. The 12.3 g of crude material obtained were  
35 purified by dry column chromatography using 300 g silica as a support. The material was eluted  
from the column using chloroform, methanol and acetic acid—94:4:2 (v/v)—as eluant and  
fractions of the eluate were collected. The fractions which contained pure material were  
combined and the eluant removed under reduced pressure. The residue was recrystallised from  
ethanol to give colourless needles. Yield 6.4 g, m.p. 230 to 233°C (with decomposition).  
40 In addition, the 17-keto structure was confirmed by infra red spectral analysis. 40

### EXAMPLE 3

#### B. Second Preparation Route

##### a) 17-keto-4 $\alpha$ ,5 $\alpha$ -epoxyandrostando[2,3-d]isoxazole (compound IX)

45 To a stirred suspension of pyridinium dichromate (236.25 g) in dichloromethane (5.625 litres)  
in a 20 litre flange top reactor was added a solution of 17 $\beta$ -hydroxy-4 $\alpha$ ,5 $\alpha$ -epoxyandros-  
tano[2,3-d]isoxazole [compound VIII(b)] (187.5 g) in dichloromethane (5 litres). A further portion  
of dichloromethane (625 ml) was added and the mixture stirred for 120 hours at room  
temperature. A thin layer chromatography (TLC) examination (see Note 1 below) of the reaction  
50 mixture at this time showed unreacted compound VIII(b) present. A further portion of pyridinium  
dichromate (30 g) was added and stirring continued overnight. A TLC examination at this point  
confirmed the absence of compound VIII(b). Alumina (261 g—Brockman grade 1) was added  
and stirring continued for a further 2 hours. The solids present in the reaction mixture were  
removed by filtration through a bed of Hyflo filter aid and the bed washed with dichloromethane  
55 (2 x 1 litres). The filtrates from the two batches were combined and reduced to about half  
volume (12 litres) under reduced pressure on a rotary evaporator at a bath temperature not  
exceeding 32°C. 55

The concentrated dichloromethane solution was divided into three 4 litre batches and each  
batch washed successively with 1 M hydrochloric acid solution (3 x 1600 ml), saturated sodium  
60 bicarbonate (3 x 1500 ml) and saturated brine (1000 ml). The washed organic layers were  
combined and dried over 500 g of Drierite (calcium sulphate). The Drierite was removed by  
filtration under suction, and washed with dichloromethane (2 x 750 ml). The filtrate and washes  
were combined and treated with activated charcoal (2 x 260 g), and then filtered through Hyflo  
filter aid with the filter aid being washed with dichloromethane (2 x 1000 ml). The filtrate and  
65 washes were combined and evaporated to dryness under reduced pressure. 65

The yield of compound IX was 339.6 g (91%).

Note 1: TLC was performed by acidification of an aliquot from the reaction mixture and extraction into ethyl acetate. Merck 60F 254 plates were employed and the solvent systems were as follows:

5	Chloroform: methanol: acetic acid	96:2:2 by volume-silica	5
	Dichloromethane: methanol	97:3 by volume-silica	
10	Chloroform: ethyl acetate	90:10 by volume-alumina	10

b) *2 $\alpha$ -cyano-4 $\alpha$ ,5 $\alpha$ -epoxyandrostan-3,17-dione (compound III)*

To a stirred suspension of the thus-prepared compound IX (325.4 g) in tetrahydrofuran (THF-12 litres) in a 20 litre flange top reactor was added a solution of potassium hydroxide (99.5 g) in THF: water (2.76 litres: 0.98 litres) and the resulting mixture stirred at room temperature for 41 hours. 15

A TLC examination at this point showed the reaction was incomplete. A further portion of THF (3 litres) was added and mixture stirred for a further 24 hours. TLC examination showed the reaction was still incomplete due to the insolubility of compound IX. To overcome this, methanol (2.5 litres) was added and the mixture stirred overnight. TLC examination then showed that the reaction had gone to completion. 20

The suspension was slowly added with stirring to distilled water (40 litres), the flask was washed with methanol 0.5 litres) and the washings added to the water. A clear solution was obtained. The pH was carefully adjusted to from 4.5 to 5.0 by slowly adding, with stirring, 1 M hydrochloric acid (about 2.5 litres). The resultant suspension was stirred out for 4 hours, then the product collected by filtration under suction to give a first crop which was washed with water (15 litres). The addition of the water to the filtrate and cooling to 10°C gave a second crop which was collected by filtration under suction and washed with water (15 litres). The two crops were dried at 60°C under reduced pressure for 18 hours. 25 30

This yield: crop 1 177.7 g  
crop 2 114.1 g

The two crops were shown by TLC examination, infra red spectral data IR and melting point (236° to 237°C with decomposition) to be pure compound III. The crops were combined to give 291.8 g (90%) of final product as a white crystalline powder, but since the product obtained was highly crystalline with a surface area below the preferred specification limit, it was reworked as described below. 35 40

c) *Controlled Precipitation of Compound III*

In order to bring the particle size of the thus-prepared compound III within the preferred specification of from about 2 to about 4 m<sup>2</sup>g<sup>-1</sup>, the compound was further treated in accordance with the method of Application No. 84-27300 as follows:

Under nitrogen, dimethylformamide (DMF-1.12 litres) at 60°C was added to a flat-top reactor charged with compound III (160 g) and the mixture stirred using a Buddeberg stirrer (200 rpm) until all solids had dissolved. 45

The solution was filtered hot, then cooled to 33°C. Deionised water (1.12 litres) was then added over 4 minutes using a peristaltic pump and the suspension stirred out for 1 hour at ambient temperature, with the stirring rate being readjusted to 200 rpm after 5 minutes since coagulation resulted in a decrease in the stirring rate. The resulting solids were collected by filtration under suction, then washed with 50% aqueous DMF (2 litres), followed by water (5.5 litres), after which they were dried under suction with the aid of a rubber dam for 2 hours. Drying was completed in vacuo after 66 hours at 700 mBar/65°C and 5h/1000 mBar/65°C to yield 150.2g (94%) of compound III as a white crystalline powder melting at 235.2° to 237°C (with decomposition). Surface area measurements (Strohlein) gave duplicate values of 2.79 m<sup>2</sup>g<sup>-1</sup> and 2.73 m<sup>2</sup>g<sup>-1</sup>, mean 2.76 m<sup>2</sup>g<sup>-1</sup>. 50 55

FORMUATIONS:

Example 4

5	<u>Capsules:</u>	<u>Formulations</u>	<u>60 mg</u>	<u>120 mg</u>	5
		Active compound III	60 mg	120 mg	
10		Lactose	86 mg	172 mg	10
		Maize starch	86.65 mg	173.3 mg	
15		Magnesium			15
		stearate	<u>2.35 mg</u>	<u>4.7 mg</u>	
			<u>235 mg</u>	<u>470 mg</u>	

Preparation

These formulations are prepared by either conventional dry blending techniques or more preferably conventional wet granulation techniques. Encapsulation is effected by means of conventional equipment.

Example 5

30	<u>Tablets</u>	<u>Formulations</u>	<u>60 mg</u>	<u>120 mg</u>	<u>240 mg</u>	30
		Active compound				
		III	60 mg	120 mg	240 mg	
35		Lactose	80 mg	160 mg	320 mg	35
		Maize starch	85 mg	170 mg	340 mg	
40		Magnesium				40
		stearate	<u>2 mg</u>	<u>4 mg</u>	<u>8 mg</u>	
45			<u>227 mg</u>	<u>454 mg</u>	<u>908 mg</u>	45

Preparation

The powdered ingredients are granulated by means of conventional wet granulation techniques. The tablets are compressed using conventional compression equipment to tablet or caplet form.

Example 6

55	<u>Suppositories:</u>	<u>Formulations</u>	<u>60 mg</u>	<u>120 mg</u>	<u>240 mg</u>	55
		Active compound				
		III	60 mg	120 mg	240 mg	
60		Suppository				60
		base	qs	qs	qs	

Preparation

The suppositories are prepared from a suitable suppository base (e.g. Suppocire, Witepsol, Novata, etc.) using conventional poured melt techniques or cold compression.

*Example 7*

5	Ointment:	Formulations	1%	2.5%	5%	5
		Active compound III	1%	2.5%	5%	
		Ointment base	to 100%	to 100%	to 100%	

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*Preparation*

The ointments are prepared by incorporating the active compound into a suitable pre-prepared ointment base (e.g. white soft paraffin).

15 *Example 8*

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20	Cream	Formulation	1%	2.5%	5%	20
		Active compound III	1%	2.5%	5%	
		Cream base	to 100%	to 100%	to 100%	

25 *Preparation*

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The creams are prepared by incorporating the active compound into a suitable pre-prepared cream base (e.g. aqueous cream B.P.).

Example 9

<u>Suspension</u>	<u>Formulations</u>	<u>1.2%</u>	<u>2.4%</u>	<u>4.8%</u>	
5	Active compound				5
	III	1.2%	2.4%	4.8%	
10	Citric acid				10
	(anhydrous) BP	0.237%	0.237%	0.237%	
15	Sorbitol				15
	solution BPC	20%	20%	20%	
20	Cetomacrogol				20
	1000 BPC	0.09%	0.09%	0.09%	
25	Sodium				25
	phosphate BP	0.63%	0.63%	0.63%	
30	Sodium carboxy-				30
	methyl cellulose	0.20%	0.20%	0.20%	
	Veegum K	1.00%	1.00%	1.00%	
35	Flavour	qs	qs	qs	35
	Preservative	qs	qs	qs	
40	Purified water	to 100%	to 100%	to 100%	40

Preparation

The suspensions are prepared by conventional manufacturing techniques having regard to the processing properties of the ingredients. Finally, each suspension is passed through a homogeniser.

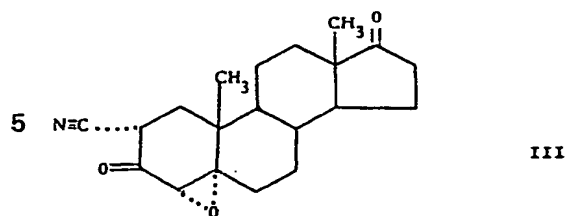
Example 10

A parenteral formulation was prepared using the following ingredients:

Ingredients	% w/v	
Active compound III	0.50	
Sodium phosphate (B.P.)	0.60	
55 Sodium chloride (Ph.Eur)	0.66	55
Potassium hydroxide	0.11	
Water for injection—balance to	100%	

## 60 CLAIMS

1. A compound of the formula:



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for use in a method of treatment of the human or animal body by therapy.

2. A compound of the formula III defined in claim 1 for use in a method of treatment of the human or animal body by therapy to modulate or inhibit adrenal steroidogenesis, in particular in therapy requiring the administration of an adrenocortical inhibitor.

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3. A compound of the formula III defined in claim 1 in particulate form consisting of particles having a mean equivalent sphere volume diameter of less than about 20 microns, at least 95% of the particles having a particle size of less than about 50 microns.

4. A compound according to claim 3, wherein the mean equivalent sphere volume diameter of the particles is from about 5 to about 10 microns.

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5. A compound of the formula III defined in claim 1 in particulate form, the particle size being such as to provide a specific surface area of at least about  $2\text{m}^2\text{g}^{-1}$ .

6. A compound according to claim 5, wherein the particle size provides a specific surface area of from about 2 to about  $5\text{m}^2\text{g}^{-1}$ .

7. A compound of the formula III defined in claim 1 in an isolated and essentially pure form.

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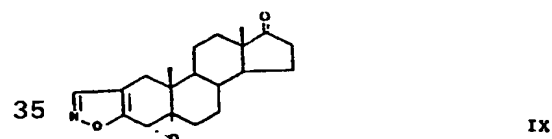
8. A compound according to claim 7 having a melting point of from about  $230^\circ$  to about  $240^\circ\text{C}$ .

9. A compound according to claim 7 or claim 8 having one or more of the characteristics defined in claims 3 to 6.

10. A process for the preparation of a compound of the formula III defined in claim 1,

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which process comprises opening the isoxazole ring of a compound of the formula:

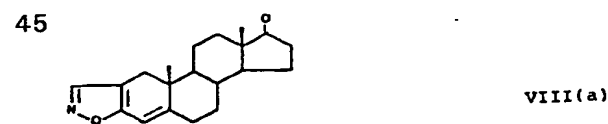


11. A process according to claim 10, wherein the ring is opened by treatment with a strong base.

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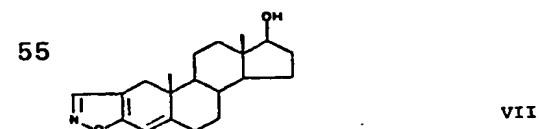
12. A process according to claim 11, wherein the base is an alkali metal hydroxide or alkoxide.

13. A process according to any one of claims 10 to 12, wherein the compound of formula IX is obtained by epoxidation of a compound of the formula:



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14. A process according to claim 13, wherein the compound of formula VIII(a) is obtained by oxidation of a compound of the formula:



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15. A process according to any one of claims 10 to 12, wherein the compound of formula IX is obtained by oxidation of a compound of the formula:

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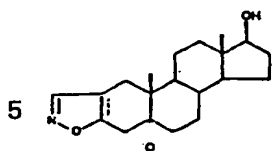
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VIII(b)

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16. A process according to claim 14 or claim 15, wherein oxidation is effected using pyridinium chlorochromate or pyridinium dichromate.
- 10 17. A process according to claim 15 or claim 16, wherein the compound of formula VIII(b) is obtained by epoxidation of a compound of the formula VII defined in claim 14. 10
18. A process according to claim 13 or claim 17, wherein epoxidation is effected in using hydrogen peroxide or a percarboxylic acid.
19. A process according to claim 10 and substantially as hereinbefore described with reference to any one of Examples 1 to 3. 15
20. A compound of the formula III defined in claim 1 when prepared by a process according to any one of claims 10 to 19.
21. A pharmaceutical composition, which composition comprises a compound according to any one of claims 3 to 8 or claim 20, and one or more pharmaceutically-acceptable excipients or carriers. 20
22. A pharmaceutical composition in unit dosage form, which composition comprises a compound of the formula III defined in claim 1 in a unit dose of from about 30 to about 250 mg.
23. A composition according to claim 22, wherein the unit dose is from about 50 to about 25 120 mg. 25
24. A composition according to claim 23, wherein the unit dose is less than about 100 mg.
25. A composition according to any one of claims 22 to 24, wherein the compound is as defined in any one of claims 3 to 8 or claim 20.
26. A composition according to any one of claims 21 to 25, wherein the active compound 30 of formula III is employed in admixture with one or more active compounds such as trilostane. 30
27. A composition according to any one of claims 21 to 26, wherein the composition is in the form of a tablet or capsule.
28. A compound of the formula III defined in claim 1 when presented in a form for use in a method of treatment of the human or animal body by therapy.
- 35 29. A package comprising a plurality of unit doses of a compound of the formula III defined in claim 1 and including instructions for use in a method of treatment of the human or animal body by therapy. 35
30. Use of a compound of the formula III as defined in any one of claims 1 to 9 for the manufacture of a medicament having enhanced activity for use in a method of treatment of the human or animal body by therapy, in particular to modulate or inhibit adrenal steroidogenesis. 40
31. A method of treatment of a patient to modulate or inhibit adrenal steroidogenesis, which method comprises administering to the patient an effective amount of a compound as defined in any one of claims 1 to 8, 20 or 28, or a composition as defined in any one of claims 21 to 27.